DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

October 23, 2017

Hologic, Inc.
Jeffrey Hergesheimer
Regulatory Affairs Specialist
10210 Genetic Center Drive
San Diego CA 92121

Re: K172282
Trade/Device Name: Panther Fusion Paraflu Assay
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: II
Product Code: OOU, OOI
Dated: July 28, 2017
Received: July 28, 2017

Dear Mr. Hergesheimer:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K172282

Device Name
Panther Fusion Paraflu Assay

Indications for Use *(Describe)*
The Panther Fusion Paraflu assay is a multiplex real-time PCR (RT-PCR) in vitro diagnostic test for the rapid and qualitative detection and differentiation of parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus and parainfluenza 4 virus (HPIV-1, HPIV-2, HPIV-3, and HPIV-4). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection.

This assay is intended to aid in the differential diagnosis of HPIV-1, HPIV-2, HPIV-3, and HPIV-4 infections in humans. Negative results do not preclude HPIV-1, HPIV-2, HPIV-3, and HPIV-4 infections and should not be used as the sole basis for treatment or other management decisions. This assay is designed for use on the Panther Fusion system.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY
Panther Fusion Paraflu Assay

I. SUBMITTER

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Date Prepared: July 24, 2017

II. DEVICE

Proprietary Name of Device:	Panther Fusion Paraflu Assay
Classification Name:	Respiratory viral panel multiplex nucleic acid assay
Regulation Number:	21 CFR 866.3980 and 862.2570
Regulatory Class:	Class II
Product Code:	OOU and OOI

III. PREDICATE DEVICE

The predicate device is the Prodesse ProParaFlu+ Assay (K153223; cleared December 9, 2015, Hologic, San Diego, CA).

IV. DEVICE DESCRIPTION

The Panther Fusion Paraflu assay is a multiplex real-time PCR (RT-PCR) in vitro diagnostic test developed for use on the fully automated Panther Fusion system to detect and differentiate parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus, and parainfluenza 4 virus directly from nasopharyngeal swab specimens.

The Panther Fusion Paraflu assay involves the following steps: Sample lysis, nucleic acid capture and elution, and multiplex RT-PCR where analytes (when present) are simultaneously amplified, detected and differentiated. Nucleic acid capture and elution takes place in a single tube on the Panther Fusion system. The eluate is transferred to the Panther Fusion system reaction tube containing the assay reagents. Multiplex RT-PCR is then performed for the eluted nucleic acid on the Panther Fusion system.

Nucleic acid capture and elution: Prior to processing and testing on the Panther Fusion system, specimens are transferred to a tube containing specimen transport media (STM) that lyses the cells, releases target nucleic acid and protects them from degradation during storage. The Internal Control-S (IC-S) is added to each test specimen and controls via the working Panther Fusion Capture Reagent-S (wFCR-S). The IC-S in the reagent is used to monitor specimen processing, amplification and detection. Magnetic particles with covalently bound oligonucleotides mediate the nucleic acid capture. Capture oligonucleotides hybridize to total nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the lysed specimen in a magnetic field. Wash and aspiration steps remove extraneous components debris from the reaction tube. The elution step elutes purified nucleic acid.

Elution transfer and RT-PCR: During the elution transfer step, eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted mastermix. Target amplification occurs via RT-PCR. A reverse transcriptase generates a DNA copy of the target sequence. Target specific forward and reverse primers and probes then amplify targets while simultaneously detecting and discriminating multiple target types via multiplex RT-PCR. The Panther Fusion system compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of the analyte. The positive result for each analyte will be accompanied by the cycle threshold (Ct) value. The analytes and the channel used for their detection on the Panther Fusion system is summarized in the table below.

Analyte	Gene Targeted	Instrument Channel
HPIV-1	Hemagglutinin neuraminidase	FAM
HPIV-2	Hemagglutinin neuraminidase	HEX
HPIV-3	Hemagglutinin neuraminidase	ROX
HPIV-4	Nucleocapsid	RED647
Internal Control	Not applicable	RED677

Assay Components

The reagents required to perform the Panther Fusion Paraflu assay are packaged and sold separately. There are 7 boxes containing 9 reagents which are required for sample processing. A description of the components that are required to perform the Panther Fusion Paraflu assay are detailed in **Table 1.** In addition, there is one ancillary kit, Panther Fusion Specimen Lysis Tubes, which is required for processing of specimens prior to testing on the Panther Fusion system.

Table 1: Reagents Required to Perform the Panther Fusion Paraflu Assay

Box	Components Description
1	Panther Fusion Paraflu Assay Cartridges
2	Panther Fusion Extraction Reagent-S • Box Contains: o Panther Fusion Capture Reagent-S o Panther Fusion Enhancer Reagent-S
3	Panther Fusion Internal Control-S
4	Panther Fusion Reconstitution Buffer I
5	Panther Fusion Elution Buffer
6	Panther Fusion Oil
7	Panther Fusion Paraflu Assay Controls • Box Contains: o Panther Fusion Paraflu Positive Control o Panther Fusion Negative Control

In addition, select components can also be ordered in the following bundles:

- Panther Fusion Universal Fluids Kit: (contains Panther Fusion Oil and Panther Fusion Elution Buffer).
- Panther Fusion Assay Fluids I-S: (contains Panther Fusion Extraction Reagents-S, Panther Fusion Internal Control-S, and Panther Fusion Reconstitution Buffer I).

Instrumentation

The Panther Fusion Paraflu assay has been designed for and validated on the Panther Fusion system. The Panther Fusion system is an integrated hardware and software system that together with the Panther Fusion Paraflu assay fully automates all the steps necessary to perform the assay.

The Panther Fusion system integrates Hologic's commercialized Panther instrument system with an add-on sidecar, the Panther Fusion module, which extends the functionality of the Panther system by increasing the assay processing capabilities to include multiplex real-time RT-PCR. The Panther Fusion module includes instrument hardware and software and can be installed on existing Panther instruments or ordered with new Panther instruments.

The Panther Fusion system employs non-specific target capture (NSTC) for the purification of RNA and DNA from the sample, followed by nucleic acid amplification and real-time fluorescent detection. The process involves sample loading and preparation (i.e. nucleic acid extraction) on the Panther instrument using the same workflow and processing steps as for other commercialized Hologic Aptima TMA assays. The extracted nucleic acid for each sample is transferred to the Panther Fusion module where PCR amplification and detection occurs.

V. INDICATIONS FOR USE

Intended Use

The Panther Fusion Paraflu assay is a multiplex real-time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus and parainfluenza 4 virus (HPIV-1, HPIV-2, HPIV-3, and HPIV-4). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection.

This assay is intended to aid in the differential diagnosis of HPIV-1, HPIV-2, HPIV-3, and HPIV-4 infections in humans. Negative results do not preclude HPIV-1, HPIV-2, HPIV-3, and

HPIV-4 infections and should not be used as the sole basis for treatment or other management decisions. This assay is designed for use on the Panther Fusion system.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

A comparison of the Panther Fusion Paraflu assay to the predicate Prodesse ProParaFlu+ (K153223) is summarized in **Table 2** (similarities) and **Table 3** (differences).

Table 2: Similarities Between Panther Fusion Paraflu Assay and Predicate Device

Item	Prodesse ProParaFlu+ Assay (Predicate Device)	Panther Fusion Paraflu Assay (Subject Device)
Technology Principle of Operation	Multiplex Real Time RT-PCR	Same
Analyte	Viral RNA	Same
Patient Population	Male and female patients with signs/symptoms of respiratory infection	Same
Specimen Types	Nasopharyngeal (NP) swab specimens	Same
Assay Controls	Internal control in each sample. External control processed at periodic intervals.	Same

Table 3: Differences Between Panther Fusion Paraflu Assay and Predicate Device

Item	Prodesse ProParaFlu+ Assay (Predicate Device)	Panther Fusion Paraflu Assay (Subject Device)
Organisms Detected	Human Parainfluenza Virus (HPIV) Types 1, 2, and 3	Same. Plus HPIV-4.
Platform	Manual multiplex real-time RT-PCR platform. Uses Roche MagNA Pure LC System or bioMerieux NucliSENS easyMAG for nucleic acid extraction and the Cepheid SmartCycler II system for real time RT-PCR	Automated multiplex real-time RT-PCR platform. Uses Panther Fusion system for all steps including nucleic acid extraction, amplification, detection and result processing.
Intended Use	The Prodesse ProParaflu+ Assay is a multiplex Real-Time PCR (RT-PCR) *in vitro* diagnostic test for the qualitative detection and discrimination of Parainfluenza 1 Virus, Parainfluenza 2 Virus and Parainfluenza 3 Virus (HPIV-1, HPIV-2 and HPIV-3) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of respiratory tract infections. This Assay targets the conserved regions of the Hemagglutinin-Neuraminidase (HN) gene of HPIV-1, HPIV-2 and HPIV-3, respectively. The detection and discrimination of HPIV-1, HPIV-2 and HPIV-3 nucleic acids from symptomatic patients aid in the diagnosis of human respiratory tract parainfluenza infections if used in conjunction with other clinical and laboratory findings. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b Viruses. Negative test results are presumptive and should be confirmed by cell culture. Negative results do not preclude Parainfluenza 1, 2 or 3 virus infections and should not be used as the sole basis for treatment or other management decisions.	The Panther Fusion Paraflu assay is a multiplex real-time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus and parainfluenza 4 virus (HPIV-1, HPIV-2, HPIV-3, and HPIV-4). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. This assay is intended to aid in the differential diagnosis of HPIV-1, HPIV-2, HPIV-3, and HPIV-4 infections in humans. Negative results do not preclude HPIV-1, HPIV-2, HPIV-3, and HPIV-4 infections and should not be used as the sole basis for treatment or other management decisions. This assay is designed for use on the Panther Fusion system.
Time to Obtain Test Results	Approximately 4 hours	Approximately 2.5 hours

VII. PERFORMANCE DATA

The following performance data were provided in support of the substantial equivalence determination.

Brief Description of Analytical (Non-Clinical) Studies

The following analytical studies (non-clinical) were conducted to support the clearance of the Panther Fusion Paraflu Assay on the Panther Fusion System.

Analytical Sensitivity and Limit of Detection (LoD) of Nasopharyngeal Swab Specimens

The LoD was determined by testing dilution panels for HPIV-1, HPIV-2, HPIV-3 and HPIV-4 made by spiking them into pooled negative NP swab clinical specimens. At least 36 replicates were tested using three reagent lots on three instruments per test concentration, per each virus types. The LoD was based on results from the lowest concentration with $\geq$ 95% positive rate. The LoD values for each virus type were verified by testing newly prepared panel for at least 20 replicates. Verified LOD concentrations are shown in **Table 4.**

Table 4: LoD Determination Panel Description

Viral Strain	LoD Concentration
HPIV-1	$1x10^{-2.0}$ TCID50/mL
HPIV-2	$1x10^{2.0}$ TCID50/mL
HPIV-3	$1x10^{1.0}$ TCID50/mL
HPIV-4	$1x10^{0.5}$ TCID50/mL

Interference

Mucin, whole blood and other potentially interfering substances (medications and over-the-counter or OTC products) that may be present in the samples were evaluated in the Panther Fusion Paraflu assay. Clinically relevant amount of the potentially interfering substances were added to simulated clinical matrix and tested unspiked or spiked with cultured HPIV-1, HPIV-2, HPIV-3 and HPIV-4 at their respective 3X LoD concentrations. The substances consisted of nasal sprays (liquid and powder), ingestible pills, lozenges, injectable and endogenous substances. No interference in performance of the Panther Fusion Paraflu assay was observed

in the presence of a representative brand of the following potentially interfering substances at the concentrations stated in **Table 5**.

Table 5: Potentially Interfering Substances

Type	Substance Name	Active Ingredient(s)	Concentration
Endogenous	Mucin	Purified mucin protein	60 µg/mL
	Human blood	Blood	2% v/v
Nasal sprays or drops	Neo-Synephrine®	Phenylephrine	15% v/v
	Anefrin	Oxymetazoline	15% v/v
	Saline	Sodium chloride	15% v/v
	Ventolin® HFA	Albuterol	15% v/v
Nasal corticosteroids	QVAR®, Beconase AQ	Beclomethasone	5% v/v
	Dexacort	Dexamethasone	5% v/v
	AEROSPAN®	Flunisolide	5% v/v
	Nasacort	Triamcinolone	5% v/v
	Rhinocort	Budesonide	5% v/v
	Nasonex	Mometasone	5% v/v
	Flonase	Fluticasone	5% v/v
Nasal gel	Zicam® (Allergy Relief)	Luffa opperculata, Galphimia, Glauca, Histaminum hydrochloricum, Sulfur	5% v/v
Throat lozenges	Chloraseptic Throat Lozenges	Benzocaine	0.63 mg/mL
		Menthol	
Anti-viral drugs	Relenza®	Zanamivir	3.3 mg/mL
	TamiFlu	Oseltamivir	25 mg/mL
	Rebitol	Ribavirin	20 mg/mL
Antibiotic, nasal ointment	Bactroban cream	Mupirocin	10 mg/mL
Antibiotic, systemic	Tobramycin	Tobramycin	4.0 µg/mL

Competitive Interference

Competitive Interference of the Panther Fusion Paraflu assay was evaluated using a simulated clinical matrix with pairs of target viruses at two different concentrations. One of the concentrations was near the Limit of Detection (3 - 5X LoD) while the other concentration was high (1000X LoD). The presence of two viruses at varying concentrations in a single sample had no effect on the analytical sensitivity (100% detection for both targets) at the concentration tested (see **Table 6**).

Table 6: Co-Infection Concentrations and Results

Condition	Target 1		Target 2		Result			
	Description	Concentration	Description	Concentration	HPIV-1	HPIV-2	HPIV-3	HPIV-4
1	HPIV-1	3X LoD	HPIV-2	1000X LoD	+	+	-	-
2	HPIV-1	3X LoD	HPIV-3	1000X LoD	+	-	+	-
3	HPIV-1	5X LoD	HPIV-4	1000X LoD	+	-	-	+
4	HPIV-2	3X LoD	HPIV-1	1000X LoD	+	+	-	-
5	HPIV-2	3X LoD	HPIV-3	1000X LoD	-	+	+	-
6	HPIV-2	3X LoD	HPIV-4	1000X LoD	-	+	-	+
7	HPIV-3	3X LoD	HPIV-1	1000X LoD	+	-	+	-
8	HPIV-3	3X LoD	HPIV-2	1000X LoD	-	+	+	-
9	HPIV-3	3X LoD	HPIV-4	1000X LoD	-	-	+	+
10	HPIV-4	3X LoD	HPIV-1	1000X LoD	+	-	-	+
11	HPIV-4	3X LoD	HPIV-2	1000X LoD	-	+	-	+
12	HPIV-4	3X LoD	HPIV-3	1000X LoD	-	-	+	+

Analytical Specificity

The analytical specificity of the Panther Fusion Paraflu assay was evaluated by testing a panel of 58 organisms, consisting of 31 viral, 26 bacterial, and 1 yeast strain representing common respiratory pathogens or flora commonly present in respiratory tract. Bacteria and yeast were tested at concentrations of 10^5 to10^8 CFU/mL or IFU/mL, except where noted. Viruses were tested at concentrations of 10^3 to 10^7 TCID50/mL. Analytical specificity of the Panther Fusion Paraflu assay was 100% for HPIV-1, HPIV-2, HPIV-3 and HPIV-4 (see **Table 7**).

Table 7: Specificity Results

Organism	Concentration	Result			
		HPIV-1	HPIV-2	HPIV-3	HPIV-4
Adenovirus 1	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
Adenovirus 7a	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
Bordetella bronchiseptica	$1x10^7$ CFU/mL	-	-	-	-
Bordetella pertussis	$1x10^8$ CFU/mL	-	-	-	-
Candida albicans	$1x10^7$ CFU/mL	-	-	-	-
Chlamydia trachomatis	$1x10^5$ CFU/mL	-	-	-	-
Chlamydophila pneumoniae (formerly *Chlamydia pneumoniae*)	$1x10^5$ IFU/mL	-	-	-	-
CMV Strain AD 169	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Coronavirus 229E	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Corynebacterium diphtheria	$1x10^7$ CFU/mL	-	-	-	-

Organism	Concentration	Result			
		HPIV-1	HPIV-2	HPIV-3	HPIV-4
Coxsackie B4	$1x10^6$ $TCID_{50}$/mL	-	-	-	-
Coxsackie B5/10/2006	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
E. coli	$1x10^7$ CFU/mL	-	-	-	-
EBV	$1x10^7$ $TCID_{50}$/mL	-	-	-	-
Echovirus 11	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
Echovirus 2	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Echovirus 3	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
Echovirus 6	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Enterovirus 68	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
Enterovirus 70	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Haemophilus Influenzae	$1x10^7$ CFU/mL	-	-	-	-
HPIV-1, C35	$1x10^2$ $TCID_{50}$/mL	+	-	-	-
HPIV-2, Greer	$1x10^2$ $TCID_{50}$/mL	-	+	-	-
HPIV-3, C243	$1x10^2$ $TCID_{50}$/mL	-	-	+	-
HPIV-4b, CH19503	$1x10^2$ $TCID_{50}$/mL	-	-	-	+
hMPV Subtype A2	$1x10^6$ $TCID_{50}$/mL	-	-	-	-
HSV-1 Macinytre Strain	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
HSV-2 Type 2G Strain	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
Influenza A (H1N1)	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Influenza A (H3N2)	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Influenza B	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Klebsiella pneumonia	$1x10^7$ CFU/mL	-	-	-	-
Lactobacillus plantarum	$1x10^7$ CFU/mL	-	-	-	-
Legionella pneumophila	$1x10^7$ CFU/mL	-	-	-	-
Measles/7/2000	$1x10^5$ $TCID_{50}$/mL	-	-	-	-
Moraxella catarrhalis	$1x10^6$ CFU/mL	-	-	-	-
Mumps virus	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Mycobacterium intracellulare	$1x10^{10}$ rRNA Copies/mL	-	-	-	-
Mycobacterium tuberculosis	$1x10^{10}$ rRNA Copies/mL	-	-	-	-
Mycoplasma pneumoniae	$1x10^6$ CFU/mL	-	-	-	-
Neisseria gonorrhea	$1x10^7$ CFU/mL	-	-	-	-
Neisseria meningitides	$1x10^7$ CFU/mL	-	-	-	-
Neisseria mucosa	$1x10^7$ CFU/mL	-	-	-	-
Polio virus	$1x10^6$ $TCID_{50}$/mL	-	-	-	-
Proteus mirabilis	$1x10^7$ CFU/mL	-	-	-	-
Proteus vulgaris	$1x10^7$ CFU/mL	-	-	-	-
Pseudomonas aeruginosa	$1x10^7$ CFU/mL	-	-	-	-
Rhinovirus 1A	$1x10^5$ $TCID_{50}$/mL	-	-	-	-

Organism	Concentration	Result			
		HPIV-1	HPIV-2	HPIV-3	HPIV-4
RSV A	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
RSV B	$1x10^4$ $TCID_{50}$/mL	-	-	-	-
Staphlycoccus aureus	$1x10^7$ CFU/mL	-	-	-	-
Staphlycoccus epidermidis	$1x10^7$ CFU/mL	-	-	-	-
Streptococcus pneumoniae	$1x10^6$ CFU/mL	-	-	-	-
Streptococcus pyogenes	$1x10^7$ CFU/mL	-	-	-	-
Streptococcus salivarius	$1x10^6$ CFU/mL	-	-	-	-
Tatlockia micdadei (formerly *Legionella micdadei*)	$1x10^7$ CFU/mL	-	-	-	-
Varicella Zoster Virus	$1x10^3$ $TCID_{50}$/mL	-	-	-	-

Carry-Over/Contamination

The carry-over/cross-contamination study was performed with negative samples alternately placed between high HPIV positive samples and tested. High positive samples were prepared by spiking HPIV-2 at 10^6 TCID50/mL (over 10,000X LoD). A total of nine separate runs with negative samples and positive samples placed in a checkerboard pattern were tested over three different instruments for a combined total of 450 positive and 450 negative samples. The carry-over rate was 0.0%.

Assay Precision

Panther Fusion Paraflu assay precision was evaluated with a 9-member panel. The panel was tested by three operators on two separate runs per day, using three reagent lots on three Panther Fusion systems over 45 days. The panel members, along with a summary of the agreement with expected results for each target is presented in **Table 8**. The mean and variability analysis between instruments, between reagent lots, between operators, between days, between runs and within runs, and overall (total) for Ct are also presented in **Table 9**.

Table 8: Percent Positive and Agreement

Target	Panel Description	% Positive	% Agreement (95% CI)
HPIV-1	3X LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	1X LoD	100.0% (160/160)	100.0% (97.7 - 100%)
	0.01X LoD	3.1% (5/161)	96.9% (92.9 - 98.7%)
	Negative	0.0% (0/162)	100.0% (97.7 - 100%)
HPIV-2	3X LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	1X LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	0.01X LoD	27.8% (45/162)	72.2% (64.9 - 78.5%)
	Negative	0.0% (0/162)	100.0% (97.7 - 100%)
HPIV-3	3X LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	1X LoD	97.5% (158/162)	97.5% (93.8 - 99.0%)
	0.01X LoD	4.9% (8/162)	95.1% (90.6 - 97.5%)
	Negative	0.6% (1/162)	99.4% (96.6 - 99.9%)
HPIV-4	3X LoD	100.0% (161/161)	100.0% (97.7 - 100%)
	1X LoD	98.1% (159/162)	98.1% (94.7 - 99.4%)
	0.01X LoD	4.3% (7/162)	95.7% (91.4 - 97.9%)
	Negative	0.0% (0/162)	100.0% (97.7 - 100%)

Table 9: Signal Variability Analysis Results

Target	Panel Member	Mean	Between Instruments		Between Reagent Lots		Between Operators		Between Days		Between Runs		Within Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPIV-1	3X LoD	35.2	0.0	0.0	0.1	0.2	0.0	0.0	0.1	0.3	0.0	0.0	0.4	1.1	0.4	1.2
	1X LoD	37.0	0.0	0.0	0.1	0.4	0.0	0.0	0.0	0.2	0.0	0.0	0.6	1.7	0.6	1.8
	0.01X LoD	42.3	0.3	0.9	0.4	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.0	0.7	1.7
HPIV-2	3X LoD	32.8	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.3	0.3	0.9	0.3	1.0
	1X LoD	34.3	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.5	1.5	0.5	1.5
	0.01X LoD	40.7	0.1	0.3	0.0	0.1	0.0	0.0	0.3	0.8	0.0	0.0	1.1	2.8	1.2	3.0
HPIV-3	3X LoD	35.5	0.5	1.4	0.0	0.0	0.0	0.0	0.2	0.7	0.0	0.0	1.5	4.4	1.6	4.7
	1X LoD	37.5	0.2	0.6	0.4	1.0	0.0	0.0	0.0	0.0	0.3	1.0	2.0	5.4	2.1	5.7
	0.01X LoD	40.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	8.3	0.7	1.7	3.4	8.5
HPIV-4	3X LoD	36.2	0.0	0.0	0.0	0.0	0.3	0.9	0.0	0.0	0.5	1.4	1.5	4.3	1.6	4.6
	1X LoD	38.1	0.0	0.0	0.0	0.0	0.2	0.7	0.0	0.0	0.0	0.0	1.9	5.0	1.9	5.1
	0.01X LoD	42.5	0.0	0.0	1.1	2.6	0.8	1.9	0.0	0.0	0.0	0.0	0.7	1.8	1.6	3.7
IC	Negative	32.1	0.0	0.0	0.0	0.1	0.0	0.2	0.0	0.0	0.1	0.5	0.4	1.2	0.4	1.4

Brief Description of Clinical Studies

Clinical testing of the Panther Fusion Paraflu assay on the Panther Fusion system included performance and reproducibility testing. Substantial equivalence is based in part on the performance study.

Clinical Performance Study

This study was performed to demonstrate clinical performance characteristics for the Panther Fusion Paraflu assay. A prospective multicenter study was conducted with leftover, remnant nasopharyngeal (NP) swab specimens from male and female individuals of all ages exhibiting signs and/or symptoms of a respiratory tract infection. Four participating US pediatric/ adolescent, private, and/or university hospitals obtained 2961 leftover, remnant NP swab specimens. The samples were tested with the Panther Fusion Paraflu assay, with reference viral culture followed by direct fluorescent antibody (DFA) identification (for HPIV-1, HPIV-2, and HPIV-3), and with 2 unique and independently developed reverse transcriptase PCR assays followed by bi-directional sequencing (PCR/sequencing, for HPIV-4). A validated PCR assay was used for discordant resolution testing for HPIV-1, HPIV-2, and HPIV-3. No discordant resolution testing was performed for HPIV-4 since reference assay was based on composite result of two different reverse transcriptase PCR assays followed by by-directional sequencing as stated above. Performance characteristics were estimated relative to valid culture/DFA or PCR/sequencing results for each sample. Sensitivity and specificity (for HPIV-1, HPIV-2, and HPIV-3) and positive and negative percent agreement (for HPIV-4) were estimated with corresponding 2-sided 95% Score CIs. Analyses were performed separately for each target analyte (HPIV-1, HPIV-2, HPIV-3, and HPIV-4).

Of the 2961 specimens, 31 specimens/samples were withdrawn, 2930 samples were processed in valid Panther Fusion Paraflu runs, 2877 (98.2%) had final valid results, and 53 (1.8%) had final invalid results. Of the 2877 samples with valid Panther Fusion results, 1359 samples were from females and 1518 samples were from males (see **Table 10**). The positivity of each analyte by age group is presented in **Table 11**.

Table 10: Summary of Subject Demographics for Prospective Samples in the Panther Fusion Paraflu Assay Evaluation

	N (%)
Total	2877 (100)
Sex	
Female	1359 (47.2)
Male	1518 (52.8)
Age Group	
0 to 28 days	82 (2.9)
29 days to < 2 years	758 (26.3)
2 to 5 years	407 (14.1)
6 to 11 years	259 (9.0)
12 to 17 years	184 (6.4)
18 to 21 years	73 (2.5)
22 to 64 years	694 (24.1)
≥ 65 years	420 (14.6)

Table 11: Panther Fusion Paraflu Positivity by Analyte and Age Group

	% Positivity (n/N)			
Analyte	**HPIV-1**	**HPIV-2**	**HPIV-3**	**HPIV-4**
All	1.5%	1.3%	2.8%	1.2%
0 to 28 days	0.0% (0/82)	0.0% (0/82)	1.2% (1/82)	0.0% (0/82)
29 days to < 2	2.1% (16/758)	2.4% (18/758)	4.4% (33/758)	1.7% (13/758)
2 to 5 years	2.5% (10/407)	2.2% (9/407)	3.4% (14/407)	2.2% (9/406)
6 to 11 years	1.6% (4/258)	0.8% (2/258)	0.4% (1/258)	2.3% (6/256)
12 to 17 years	1.7% (3/181)	3.3% (6/181)	1.1% (2/181)	0.5% (1/184)
18 to 21 years	0.0% (0/73)	0.0% (0/73)	2.7% (2/73)	0.0% (0/73)
22 to 64 years	0.7% (5/692)	0.0% (0/692)	2.2% (15/692)	0.4% (3/692)
≥ 65 years	1.2% (5/419)	0.5% (2/419)	2.9% (12/419)	0.5% (2/419)

Of the samples with valid Panther Fusion Paraflu results, 7 samples with invalid culture/DFA results and 7 samples with invalid PCR/sequencing results were excluded from the performance analyses, leaving 2870 samples evaluable for analyses for each analyte.

Of the 2870 evaluable samples tested using the Panther Fusion Paraflu assay, 1.5% (43/2870) were positive for HPIV-1, 1.3% (37/2870) were positive for HPIV-2, 2.8% (80/2870) were positive for HPIV-3, and 1.2% (34/2870) were positive for HPIV-4. Performance characteristics for detection of HPIV-1, HPIV-2, HPIV-3, and HPIV-4 in prospective NP samples were calculated (see **Table 12**).

Table 12: Panther Fusion Paraflu Assay Performance Relative to Reference Testing

Analyte	N	TP	FP	TN	FN	Prevalence[1] (95% CI)[2]	Sensitivity/PPA[3] (95% CI)[2]	Specificity/NPA[3] (95% CI)[2]
HPIV-1	2870	33	10[4]	2826	1[4]	1.2 (0.8-1.7)	97.1 (85.1-99.5)	99.6 (99.4-99.8)
HPIV-2	2870	22	15[5]	2831	2[5]	0.8 (0.6-1.2)	91.7 (74.2-97.7)	99.5 (99.1-99.7)
HPIV-3	2870	52	28[6]	2788	2[6]	1.9 (1.4-2.4)	96.3 (87.5-99.0)	99.0 (98.6-99.3)
HPIV-4	2870	29	5[7]	2835	1[7]	1.0 (0.7-1.5)	96.7 (83.3-99.4)	99.8 (99.6->99.9)

FN=false negative, FP=false positive, NPA=negative percent agreement, PPA=positive percent agreement, TP=true positive, TN=true negative

[1]Study prevalence reported, [2]Score Confidence Interval, [3]PPA and NPA apply to HPIV-4

[4]8/10 false positive results were confirmed positive and 1/1 false negative result was confirmed negative for HPIV-1 by PCR

[5]14/15 false positive results were confirmed positive and 2/2 false negative results were confirmed negative for HPIV-2 by PCR

[6]26/28 false positive results were confirmed positive and 2/2 false negative results were confirmed negative for HPIV-4 by PCR

[7]No discordant resolution testing were performed for the 5 false positive and 1 false negative results for HPIV-4

Reproducibility

Panther Fusion Paraflu assay reproducibility was evaluated at three US sites using nine panel members. Testing was performed using one lot of assay reagents and six operators (two at each site). At each site, testing was performed for at least five days. Each run had three replicates of each panel member.

A negative panel member was created using a matrix of simulated nasal swab specimen in viral transport medium (VTM). Positive panel members were created by spiking 1-2X LoD (low-

positive) or 2-3X LoD (moderate-positive) concentrations of the target analyte into a matrix of simulated nasal swab specimen, composed of cultured human cells suspended in VTM.

The agreement with expected results was 100% in the negative and moderate positive panel members and ≥96.6% in low-positive panel members for HPIV-1, HPIV-2, HPIV-3, and HPIV-4 as shown in **Table 13**.

Table 13: Agreement of Panther Fusion Paraflu Assay Results With Expected Results

Panels			Expected Results HPIV-				Agreement with Expected Results							
							HPIV-1		HPIV-2		HPIV-3		HPIV-4	
Description	**Comp.**	**Conc. ($TCID_{50}$/mL)**	**1**	**2**	**3**	**4**	**N**	**% (95% CI)**	**N**	**% (95% CI)**	**N**	**% (95% CI)**	**N**	**% (95% CI)**
HPIV-1 Low Pos	1-2X LoD	1.00E-02	+	-	-	-	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)
HPIV-1 Mod Pos	2-3X LoD	3.00E-02	+	-	-	-	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
HPIV-2 Low Pos	1-2X LoD	1.00E+02	-	+	-	-	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)
HPIV-2 Mod Pos	2-3X LoD	3.00E+02	-	+	-	-	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
HPIV-3 Low Pos	1-2X LoD	1.00E+01	-	-	+	-	87/87	100 (95.8-100)	87/87	100 (95.8-100)	86/87	98.9 (93.8-99.8)	87/87	100 (95.8-100)
HPIV-3 Mod Pos	2-3X LoD	3.00E+01	-	-	+	-	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
HPIV-4 Low Pos	1-2X LoD	3.16E+00	-	-	-	+	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)	84/87	96.6 (90.3-98.8)
HPIV-4 Mod Pos	2-3X LoD	9.49E+00	-	-	-	+	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)
Neg	N/A	N/A	-	-	-	-	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)

Comp.=composition, Conc.=concentration, CI=Score confidence interval, Mod=moderate, N/A=not applicable, Neg=negative, Pos=positive, $TCID_{50}$/mL=50% tissue culture infective dose (measure of virus titer)

The total HPIV-1, HPIV-2, HPIV-3, and HPIV-4 signal variability measured as %CV ranged from 1.11% to 5.88% in low and moderate positive panel members. For the sources of variation except the 'within-run' factor, %CV values were ≤1.40% as shown in **Table 14**.

Table 14: Signal Variability of the Panther Fusion Paraflu Assay by Panel Member

			Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Panel Description	N	Mean Ct	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPIV-1 Low Pos	88	37.2	0.0	0.0	<0.1	0.26	<0.1	0.21	<0.1	<0.1	0.79	2.13	0.80	2.16
HPIV-1 Mod Pos	89	35.3	0.18	0.52	0.0	0.0	0.11	0.31	<0.1	<0.1	0.54	1.54	0.59	1.66
HPIV-2 Low Pos	87	34.4	0.0	0.0	0.0	0.0	0.13	0.38	<0.1	<0.1	0.49	1.43	0.51	1.48
HPIV-2 Mod Pos	89	32.7	<0.1	0.16	<0.1	0.24	0.0	0.0	0.0	0.0	0.35	1.07	0.36	1.11
HPIV-3 Low Pos	86	37.8	0.14	0.37	0.31	0.81	0.0	0.0	0.0	0.0	1.81	4.78	1.84	4.87
HPIV-3 Mod Pos	89	35.5	0.0	0.0	0.49	1.40	0.0	0.0	0.0	0.0	1.83	5.17	1.90	5.36
HPIV-4 Low Pos	84	38.5	0.0	0.0	0.0	0.0	0.52	1.35	<0.1	<0.1	2.20	5.72	2.26	5.88
HPIV-4 Mod Pos	88	36.0	0.0	0.0	0.39	1.08	0.0	0.0	0.0	0.0	1.60	4.44	1.65	4.57

Ct=threshold cycle, CV=coefficient of variation, Mod=moderate, Pos=positive, SD=standard deviation
Note: In case variability from some factors may be numerically negative, SD and CV are shown as 0.0.

The signal variability as measured as %CV was ≤3.01% between sites, between operators, between days, or overall for the Panther Fusion Paraflu assay positive control (see **Table 15**).

Table 15: Signal Variability of the Panther Fusion Paraflu Assay Controls

				Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Control	Analyte	N	Mean Ct	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
Pos	HPIV-1	30	34.0	0.0	0.0	<0.1	<0.1	0.21	0.62	0.0	0.0	0.43	1.28	0.48	1.42
	HPIV-2	30	32.2	0.0	0.0	0.0	0.0	<0.1	0.26	0.0	0.0	0.28	0.88	0.30	0.92
	HPIV-3	30	32.8	0.21	0.64	0.0	0.0	0.0	0.0	0.0	0.0	0.34	1.05	0.40	1.23
	HPIV-4	30	36.1	0.0	0.0	0.0	0.0	0.81	2.24	0.0	0.0	0.73	2.01	1.09	3.01

Ct=threshold cycle, CV=coefficient of variation, Pos=positive, SD=standard deviation
Note: In case variability results from some factors are numerically negative, SD and CV are shown as 0.0.

VIII. CONCLUSIONS

The analytical and clinical study results demonstrate that the Panther Fusion Paraflu assay on the Panther Fusion system performs comparably to the predicate device that is currently marketed for the same intended use. Hardware and software verification and validation demonstrate that the Panther Fusion Paraflu assay on the Panther Fusion system will perform as intended.